

LISTING OF CLAIMS

This listing of claims will replace all prior versions of claims in the application:

1. (Original) A method for establishing undifferentiated human embryonic stem cells, comprising the steps of:
 - (a) thawing a cryopreserved human blastocyst embryo; and
 - (b) culturing at least a portion of said human blastocyst embryo on a medium capable of sustaining undifferentiated embryonic stem cells, whereby undifferentiated human embryonic stem cells are established.
2. (Original) The method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said human blastocyst embryo comprises a sphere of cells with an outer cell layer, a fluid filled cavity, and the inner cell mass.
3. (Original) The method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said human blastocyst embryo comprises a human embryo that was cryopreserved from about 5 days to about 6 days after fertilization of said embryo.
4. (Original) The method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said human blastocyst embryo has been cryogenically stored for more than four years.
5. (Original) The method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said thawing step comprises:
 - (a) a first step of treating said cryopreserved human blastocyst embryo with a first solution comprising human follicular fluid and cryoprotectant;
 - (b) a subsequent second step of treating said cryopreserved human blastocyst embryo with a second solution comprising human follicular fluid and cryoprotectant; wherein said second solution comprises a decreased concentration of cryoprotectant relative to said first solution.
6. (Original) The method for establishing undifferentiated human embryonic stem cells of claim 5, wherein said cryoprotectant is selected from the group consisting of sucrose, glycerol and a combination of sucrose and glycerol.
7. (Original) The method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said thawing step consists of:
 - (a) a first step of treating said cryopreserved human blastocyst embryo with a first solution comprising human follicular fluid and cryoprotectant;

(b) a subsequent second step of treating said cryopreserved human blastocyst embryo with a second solution comprising human follicular fluid and cryoprotectant;

(c) a subsequent third step of treating said cryopreserved human blastocyst embryo with a third solution comprising hFF and cryoprotectant;

(d) a subsequent fourth step of treating said cryopreserved human blastocyst embryo with a fourth solution comprising hFF and cryoprotectant; wherein said fourth solution comprises a decreased concentration of cryoprotectant relative to said third solution, said third solution comprises a decreased concentration of cryoprotectant relative to said second solution, and said second solution comprises a decreased concentration of cryoprotectant relative to said first solution.

8. (Original) The method for establishing undifferentiated human embryonic stem cells of claim 5, further comprising a subsequent third step of treating said cryopreserved human blastocyst embryo with a third solution comprising hFF and cryoprotectant; wherein said third solution comprises about 0.1-2 vol % glycerol, said second solution comprises about 2-4 vol % glycerol, and said first solution comprises about 4-6 vol % glycerol.

9. (Original) The method for establishing undifferentiated human embryonic stem cells of claim 5, wherein at least one of said treating steps is carried out for about 4-6 minutes.

10. (Original) The method for establishing undifferentiated human embryonic stem cells of claim 5, wherein said first solution and said second solution each comprise about 15-25% human follicular fluid.

11. (Original) The method for establishing undifferentiated human embryonic stem cells of claim 1, further comprising a step of removing trophectoderm from said embryo using anti-human lymphocyte antibody.

12. (Original) The method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said portion of said embryo comprises the inner cell mass.

13. (Original) An undifferentiated human embryonic stem cell culture formed using the method of any one of claims 1 to 12.

14. (Original) A method for establishing undifferentiated human embryonic stem cells comprising the steps of:

(a) obtaining a population of cryogenically stored human embryos, wherein said population of embryos consists of embryos in the blastocyst phase;

(b) thawing one or more of said embryos; and

(c) culturing at least a portion of each of said one or more thawed embryos on a medium capable of sustaining undifferentiated embryonic stem cells; whereby undifferentiated human embryonic stem cells are established.

15-24 (Cancelled)

B1 25. (Currently Amended) An undifferentiated human embryonic stem cell culture formed ~~using the method of claim 24 by~~ culturing at least a portion of an inner cell mass of a human blastocyst embryo, which is isolated by treating a cryopreserved and thawed embryo with an anti-human lymphocyte antibody, on a medium capable of sustaining undifferentiated embryonic stem cells, whereby undifferentiated human embryonic stem cells are established.

[26-31. (Cancelled)]
